Dr. Thomas Jukes Space Science Board Summer Study 1964 800 Welch Road Palo Alto, California 94304

Dear Tom:

The letter from Joshua Lederberg reached me at a most awkward time, between the Gordon Conference and the International Congress of Biochemistry. I am just back from the latter and will leave on my vacation tomorrow. I am sure that this reply will not be very profound but rather than delay longer I will send along my first thoughts.

## Autotrophs

I suppose the easiest forms of life to search for are the autotrophs. It should be possible to introduce a soil sample into a closed system containing NaHC $^{14}$ O<sub>3</sub> and determine whether there is any utilization of the C $^{14}$ O<sub>2</sub>. The larger the sample of soil the better. I would add nothing to it except the small amount of C $^{14}$ O<sub>2</sub> together with the martian atmosphere and under conditions as closely simulating those of Mars as possible. Considerable time should be allowed, perhaps several weeks, before the products are analyzed. These could be separated into volatile and nonvolatile products by use of a vacuum system with traps.

## Volatile Products

The soil sample should be made acid with dilute sulfuric acid and the gas caused to flow through a solid scintillating counter to determine if there has been any conversion of the C<sup>14</sup>O<sub>2</sub> to a nonvolatile components (original radioactivity - final volatile radioactivity). The gas should then flow through a trap with a temperature near that of dry ice. This would freeze out most of the organic volatile products. Their radioactivity could then be assayed and their identity might be determined by then passing the vapor into a mass spectrometer if such is to be on the space ship.

The gas which is not condensed in the cold trap should next be treated to remove the  $C^{14}O_2$  by passing it through ascarite or some such absorbent. A counter should be distal to this absorbant to determine if there are any volatile products such as methane, or other hydrocarbons which were not trapped by the cold trap or ascarite.

If a cold trap is not feasible, perhaps concentrated sulfuric acid could be used to absorb volatile organic compounds other than C<sup>14</sup>O<sub>2</sub>.

## Nonvolatile Compounds

The simplest procedure would be to burn the soil sample to determine if any nonvolatile- $C^{14}$  compounds are present.  $CuO_2$  at high temperature could be used or perhaps a chromic acid solution. The resulting gas should be passed through a train such as I previously described to determine if any organic compounds have been burned to  $C^{14}O_2$ .

Alternatively the sample might be pyrolyzed and the resulting gasious products trapped and analyzed for radioactivity and then be identified by mass analysis.

More elaborate tests could be devised such as successive extraction with solvents, for example benzene, alcohol and water. The extracts could then be passed through cation and anion exchange resins for further characterization and the radioactivity of the fractions estimated directly or after evaporation and then combustion to  $C^{16}O_2$ .

A control should be run on the soil prior to incubation to detect any radioactive compounds which might be present in the original soil.

Tracers other than  $C^{14}O_3$ . Other tracers such as trituin might be used or  $N^{15}$ , if a mass spectrometer is to be available. The question of nitrogen fixation might be investigated by searching for formation of  $N^{15}H_3$  or  $N^{15-}$  amino acids after subjecting the soil to Keldahl digestion. However the sensitivity of the  $N^{15}$  tracers is less than  $C^{14}$  and more complex.  $S^{35}$  might be used for a search for the sulfur becteria.

## Heterotrophs

The search for heterotrophs is perhaps more complex since there are vast array; of possible substrates and products. My suggestion would be to use a complex substrate such as a digest of algae which had been grown on  $C^{16}O_2$  and to search for the formation of volatile products as described above under the autotrophs. The incubation should be with a plentiful supply of soil and for a considerable period of time. The search for a formation of nonvolatile components would be more complex but should not be impossible. It could be done by extraction with selective solvents and by paper chromatography followed by scanning for radioactivity to determine if the finger print had been altered. The control would be the  $C^{16}$  algae digest with soil added but without incubation.

These ere ideas I am sure. Tom, are obvious to all but came to mind in the short time available to me now. Sorry I am not of more help. Best regards.

Sincerely yours,

Harland & Wood HARLAND G. WOOD

HGW:drc

ce: Dr. Joshua Lederberg